

Docket No.: 0259-0411PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Maurizio Dalle CARBONARE et al.

Application No.: 10/019,387

Confirmation No.: 6340

Filed: March 26, 2003

Art Unit: 1612

For: USE OF HYALURONIC ACID DERIVATIVES
FOR THE PREPARATION OF
PHARMACEUTICAL COMPOSITIONS AND
BIOMATERIALS FOR THE PREVENTION OF
THE FORMATION AND URE OF
CUTANEOUS SCARS

Examiner: S. Maewall

DECLARATION UNDER 37 CFR 1.312

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Anna Maria Zanellato, do hereby declare the following:

1. Attached is a copy of my *curriculum vitae*.
2. I am working as Scientific Assistant to the Patent Department at Fidia Farmaceutica and I have worked in the field of cellular biology for 13 years.
3. I am familiar with the above referenced patent application, as well as the development, usages and properties of hyaluronic acid derivatives and their uses, in particular to reducing normotrophic scarring.
4. I have read and understand the subject matter of the Office Action of September 04, 2008.

5. The following experimental reports and comments are offered in support of the patentability of the instant invention.

6. I have attached as Attachment 1 the results of a study conducted to evaluate the effect of different Hyaluronic Acid formulations on wound healing, particularly to compare results obtained by using the products described in Della Valle U.S. Patent 5,658,331 (claim 1, claim 12, and Examples 1-3) with results obtained by the present invention (Specification, page 28, line 10, referencing U.S. 4,851,521).

8. *Experimental Report of Attachment 1* - The report of Attachment 1 describes the test procedures and results for experiments conducted to evaluate the effect of HA formulations on cutaneous scarring. Treatment with the 75 % of benzyl ester product in the form of non-woven fabric resulted in unexpectedly reduced scarring as compared to treatment with a total benzyl ester (100%) in form of perforated membrane without cells (as in Della Valle prior art) (Attachment 1 to this Declaration) or the ethyl ester as described in *Davidson* or with hyaluronic acid (Attachment 1 to Zanellato Declaration 2). The results using the 100% benzyl ester of hyaluronic acid are particularly summarized in the graph of Attachment 1, from which it can be seen that as early as day 14 the scarred areas of the treatment groups (i.e. the one treated with the 75% benzyl ester according to the present invention) were at least 40% or 50% less extensive (see also day 42) than the control untreated areas. In contrast, the wounds treated with a total benzyl ester (100%) in form of perforated membrane without cells (as in Della Valle reference) were at most 5-8% less extensive than the control untreated areas. This means that scarring in the groups treated with a total benzyl ester was approximately 7 times less effective than those groups treated with the 75% benzyl ester of hyaluronic acid. .

9. Contrary to the results reported above, I would have expected the test compositions of the present invention to have merely the same activity as the control compositions. Della Valle discloses a total benzyl ester of hyaluronic acid. Comparing this composition to the claimed

composition in a cutaneous scarring model shows that the presently claimed composition was 7 times more effective than the Della Valle composition.

Furthermore, based on Della Valle, the person skilled in the art would not apply a composition without cells seeded onto it. Della Valle teaches that a membrane of 100% of hyaluronic benzyl ester is usable as artificial skin **when seeded with epithelial cells** (column 5, lines 30-41). Once implanted on the lesion area, the keratinocyte colonies create growth nuclei of rapid-growing epithelial tissue (column 5, lines 46-49). Therefore, the person skilled in the art would think that the presence of cells is very important for healing process, and if the epithelial cells are not seeded and grown in the membrane, all support of hyaluronic derivative **can not be used to enhance the healing process**.

Therefore, my expectation would have been that the scarring process would be seriously compromised. However, unexpectedly, the presently claimed invention, i.e. non-woven fabric made of Hyaff 11p75 used without cells seeded and grown in this hyaluronan derivative support, showed a 40-50% reduction of the scarred areas, **enhancing the healing process**.

I provide pictures of Hyaluronic acid derivative made in form of non-woven fabric with cells (Figure 1) or without cells (Figure 2) seeded and grown in this biomaterial. Non-woven fabric is made by Hyaff 11p75. The last picture (Figure 3) shows keratinocytes seeded and grown in the total benzyl ester in form of perforated membrane as described by Della Valle reference. A comparison demonstrates two types of cells growths : tridimensional (i.e. the type of growth of non-woven fabric) vs bidimensional growth (i.e. the type of growth of membrane). Cells cultured in two dimensions do not mimic the cell growth fashion in vivo, while cells cultured in 3 dimensional system show different morphology than those cultured in 2 D. These cells cultured in 3D show increased proteoglycan synthesis compared to monolayer (2D) grown cells (Figure 4 vs Figure 3: in Figure 4 proteoglycans of extracellular matrix are the gelatinoseous material between the cells, that are the dark fuzzy spots on the fiber of the non-woven fabric made of benzyl ester of hyaluronic acid).

In vitro animal cell growth in 3D promotes normal epithelial polarity and differentiation. Cells move and divide more quickly and have a characteristically asymmetric shape compared with that of cells in living tissue.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated:

November 25, 2009

By 
Anna Maria Zanellato

CURRICULUM VITAE
Anna Zanellato

I, Anna Zanellato, being duly sworn, depose and say that:

- I am an Italian citizen residing at Bovolenta, Padua, Italy
- I am familiar with the English language.

I further declare that:

- I graduated in Biology at the University of Padua in the academic year 1987
- I am author of 19 scientific publications.

Previous job experience:

- From 1987 to 1990 I worked at the University Department of General Pathology as researcher, where I was involved in a study pertaining to smooth muscles cell cultures; moreover I studied the variations in myosin compositions that occur in situations of vascular pathologies such as Hypertension and Atherosclerosis.
- In the years 1990-2001, I worked at Fidia farmaceutici as senior researcher and my research activity involved: analysis of the action mechanism of various trophic factors of the central nervous system; studies utilising neuronal cultures to select new, pharmacologically active, chemical molecules to prevent different types of neuronal pathologies; other studies concerning the growth and proliferation of bovine, rabbit, human, mesenchymal/articular/fibroblastic cell cultures on biomaterials.

Current job:

- I am working as Scientific Assistant to the Fidia farmaceutici Patent Department, Italy.

Hyaluronic acid derivatives in wound animal model

New experiments have been conducted to evaluate the effect of different HA derivatives in decreasing the area of cutaneous scarring in a wound animal model. The tested biomaterials are the benzyl ester of hyaluronic acid with 75% esterification (Hyaff 11p75) in form of non-woven fabric (as per Dalle Carbonare) vs the benzyl ester of hyaluronic acid with 100% esterification (Hyaff p100) in form of perforated membrane (as per Della Valle '331).

The animals were sedated by intramuscular injection of ketamine/xilazine (0,1 mg/g). The backs of the animals were shaved, washed and disinfected with chlorhexidine and iodate solution.

Four full-thickness wounds were performed on each animal using a punch with a 6 mm diameter.

Tested biomaterials:

1. Partial benzyl ester of hyaluronic acid **Hyaff 11p75** in form of non-woven fabric (US 4,851,521- EP216453)
2. Total benzyl ester of hyaluronic acid **Hyaff** in form of perforated membrane (US 5,568,331) without cells

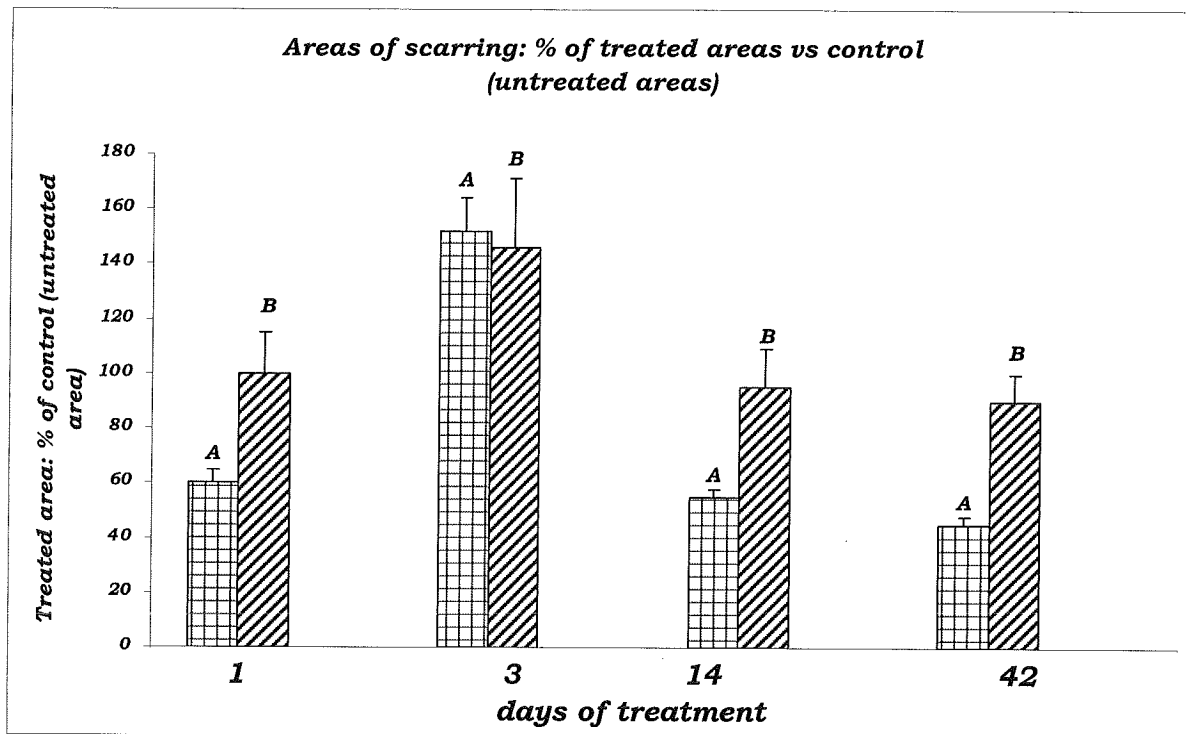
Treatment of wounds:

Groups	Number of treated sites	Treatment
A	12	1. Partial benzyl ester of hyaluronic acid, Hyaff 11p75 in form of non-woven fabric
B	12	2. Total benzyl ester of hyaluronic acid, Hyaff 11p100 in form of perforated membrane

Two wounds in each animal were treated by a **single application** of tested biomaterial, and **two were used as control, i.e. untreated sites**.

Three treated areas for group were removed at set times (1, 3, 14, 42 days). The samples were cut into sections and stained with Mallory's triple stain; the sections were analysed by optical microscope and the **scarred areas were measured**.

The graph reports values expressed as percentages of scar area of the treated sites compared to that of the untreated sites, and each value corresponds to the mean of four determinations on two different animals.



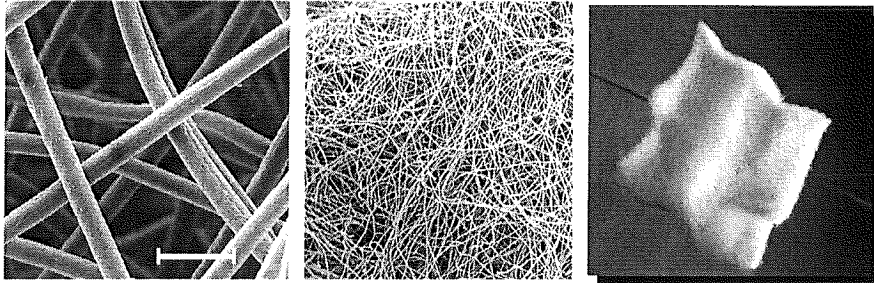
- A** Partial benzyl ester of hyaluronic acid, **Hyaff 11p75**, in the form of non-woven fabric
- B** Total benzyl ester of hyaluronic acid, **Hyaff 11p100**, in form of perforated membrane

Figure 1



Figure 2

HYAFF 3D matrix- increasing magnification



HYAFF 11 p75 non-
woven

fiber diameter: 10 micron

basis weight: 120 g/m₂

thickness: 2 mm

Figure 3



Figure 4

